# 14N NMR Spectra Sensitive to Membrane Curvature and Segmental Motions of Phospholipid Headgroup (INTERFACE SCIENCE-Solutions and Interfaces)

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# $^{14}$N NMR Spectra Sensitive to Membrane Curvature and Segmental Motions of Phospholipid Headgroup

**Emiko Okamura, Chihiro Wakai, Nobuyuki Matubayasi, and Masaru Nakahara**

Surface curvature dependence of the dynamical structure of the headgroups of phospholipids was studied by $^{14}$N NMR on liposomes and micelles. When the diameter was increased from 55 to 100 nm in unilamellar vesicles, $^{14}$N NMR signals, for the first time observed for the micelles and unilamellar vesicles with high surface curvatures, suddenly vanished.

*Keywords* : Bilayer/ Liposome/ Micelle/ Dynamics/ Quadrupole

Molecular level study of the structure and dynamics of phospholipid membranes is of great importance for a better understanding of various biomembrane functions. In order to elucidate the correlation between the dynamical structures and functions of the membranes at an atomic-site level, we now apply multinuclear NMR spectroscopy to model membranes like phospholipid liposomes, micelles, and emulsions. We especially pay attention to the membrane curvature dependence of the dynamical structures of these membranes. This is because the membrane curvature can be an important factor controlling biomembrane function and integrity. For example, plasma lipoproteins regulate apoprotein binding in the metabolism process by controlling their surface curvatures. Morover, we think the molecular level study of the curvature effect is also crucial for the potential utilization of the functions of the liposomes and emulsions such as solubilization, drug delivery, and so on.

The effect of the curvature is expected to be most significant for hydrophilic headgroups at surfaces. In plasma lipoproteins described above, lipid headgroups at surfaces are considered to play an important role for the apoprotein binding. So far, however, there have been no systematic studies of the curvature effect. We first succeeded in applying $^{14}$N NMR to probe the surface curvature dependence of the fluctuation of the headgroup structure [1].

We focus on uni- (**I**, **II**) and multilamellar liposomes (**III**, **IV**) of 1,2-dipalmitoylphosphatidylcholine (DPPC) with different diameters from 55 nm (**I**) to 1-30 μm (**IV**). The schematic structure of the liposome (**I**) is depicted in Figure 1. We also compare the $^{14}$N NMR spectra with those of the spherical micelles of 1-palmitoyl-lysophosphatidylcholine (PaLPC) with diameter of 5 nm as given in Figure 2.

Figure 3 shows the $^{14}$N NMR spectra in four types of liposomes. The spectra of aqueous phosphorylcholine and PaLPC solutions are also given. A sharp symmetric peak

## INTERFACE SCIENCE — Solutions and Interfaces —

*Scope of research*

*Structure and dynamics of a variety of ionic and nonionic solutions of physical, chemical, and biological interests are systematically studied by NMR under extreme conditions. High pressures and high temperatures are employed to shed light on microscopic controlling factors for the structure and dynamics of solutions. Vibrational spectroscopic studies are carried out to elucidate structure and orientations of organic and water molecules in ultra-thin films. Crystallization of protein monolayers, advanced dispersion systems at liquid-liquid interfaces, and biomembranes are also investigated.*

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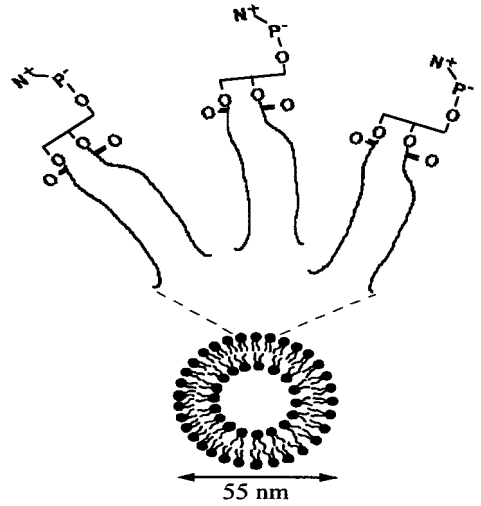


**Figure 1.** Schematic structure of the unilamellar liposome (**I**).

at -329 ppm is assigned to the choline $N^+$-methyl group (a, b). The $^{14}N$ NMR spectra of the DPPC liposomes depend remarkably on the surface curvature and temperature. This is most evident for the highly curved liposomes (**I**) (traces c-f). The observed chemical shifts are, however, the same as those of the isotropic (a) and micellar solution (b). Further, the increase in the liposome diameter from 55 (**I**) to 100 nm (**II**) strongly affects the spectral feature of the choline signal (g).

The chemical shift values indicate the conformation of the choline $N^+$-methyl sites are essentially similar. This is quite reasonable, considering that the choline $N^+$-methyl groups are surrounded by many water molecules and that the conformation is mainly dominated by the hydration force irrespective of the surface curvature.

The rotational motion of the choline $N^+$ is restricted and that the fluctuation of the P-N vector is permitted only in the direction along the molecular axis (parallel to the chain axis) in the liposomes with small curvatures. Note that the appearance of the NMR signal is only due to the rotational motion of the quadrupole $^{14}N$ nucleus inducing orientation relaxation.

We discuss the dynamics of the headgroup choline $N^+$ site. We strictly measure the spin-lattice and spin-spin relaxation times ($T_1$ and $T_2$, respectively) of the liposomes (**I**) and the micelles. As a result, we succeed in the unique determination of the rotational correlation time ($\tau$) and the quadrupolar coupling constant ($QCC$) from the following two equations (where $\omega=2\pi$ 19 MHz):

$$\frac{1}{T_1} = \frac{3\pi^2}{10}(QCC)^2\,\tau\left(\frac{1}{1+\omega^2\tau^2} + \frac{4}{1+4\omega^2\tau^2}\right)$$

$$\frac{1}{T_2} = \frac{3\pi^2}{20}(QCC)^2\,\tau\left(3 + \frac{5}{1+\omega^2\tau^2} + \frac{2}{1+4\omega^2\tau^2}\right).$$

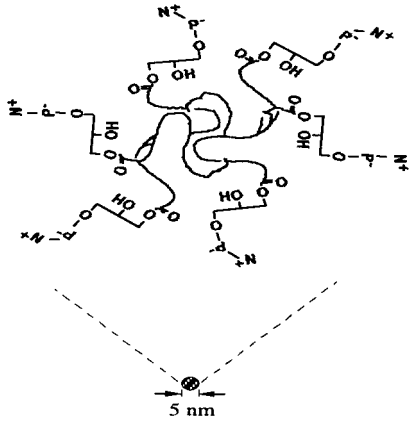


**Figure 2.** Schematic structure of the PaLPC micelle.

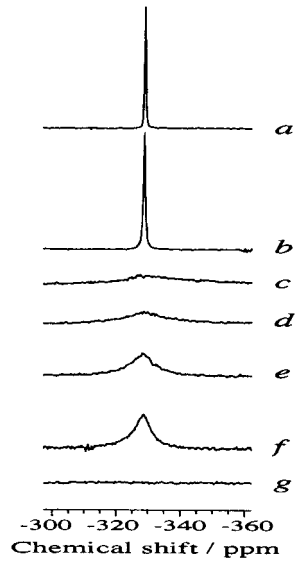


**Figure 3.** $^{14}N$ NMR spectra of the DPPC liposomes. For comparison, the spectra of aqueous phosphorylcholine and micellar PaLPC solutions at 30 ˚C are given by the traces a and b, respectively. The traces c, d, e, and f denote the spectra of the liposomes (**I**) at 30, 36, 40, and 50 ˚C, respectively; the trace g representing the spectra of the liposomes (**II**-**IV**) at 50 ˚C. Saturated $KNO_3$ in $D_2O$ was used as an external reference.

It is concluded that the surface curvature dominates the segmental motions of the hydrophilic choline groups. The $^{14}N$ NMR spectroscopy is, therefore, proven to sensitively reflect surface curvature dependence in bilayers and micelles. Molecular level studies of the dynamical structure of the lipoprotein surfaces and the apoprotein binding mechanisms are now in progress, by combining site-selective NMR and the surface curvature effect.

## Reference

1. E. Okamura et al., *Chem. Lett.*, **1997**, 1061-1062 .